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Simultaneous and rapid separation and determination of vitamins b1, b2, pp, b6 by using ion-pair rp-hplc and ultra violet detection in multivitamin tablets

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ABSTRACT

Aim: This study was aimed to evaluate simultaneous and rapid separation and determination of vitamins b1, b2, pp, b6 by using ion-pair rp-hplc and ultra violet detection in multivitamin tablets.

Method and Materials: The HPLC system (shimadzu-japan) consist of control unit (scl-10-A vp) , oven(CTO-10A vp) and two types of detectors: Ultra violet Detector (SPD-10AV vp), fluorescent Detector (RF-10AXL), degassing unit (DGU-14A) isocratic pump, with valve 20-µL loop, (LC-solution) software was used for collected data, Chromatographic analysis was performed on RP-C18 (250 mm x4. 6 mm, 5 µm particle size) column.

Results: Simultaneous determination of B1, B2, B3, B6 vitamins were obtained with above mobile phase where the pH was adjusted to 5 ± 0.2 by using 2N sodium hydroxide solution to prevent peaks interfering during separation the mixture of studied compounds, mobile phase was filtered through 0. 45µm Millipore filter, flow rate 1ml/min and octadecyle column ODS C18 (250x4. 6,5µm), measurements were made at λ=280 nm..

Conclusion: It was concluded that simultaneous determination of the four water-soluble vitamins thiamine hydrochloride, riboflavin, nicotinamide and pyridoxine hydrochloride was performed on column of c18(250x4. 6,5µm), a mixture of (water: methanol: glacial acetic acid) ratio(72:27:1 v/v) as the mobile phase with flow rate of 1mL/men, the effluent was monitored at 280 nm.

Keywords: HPLC, multivitamin preparations, niacinamide, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride.

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Introduction

Vitamins of group B belong to the most important biologically active substances, as these ensure the normal performance of human body through participation in the biosynthesis of proteins and functioning of the central nervous, cardiovascular and gastrointestinal system. Their input into the body with plant items or food of animal origin, some of them are synthesized by intestinal microflora. Most of the group B vitamins are present in food of plant origin: cereals, bran, rough flour, yeast and lesser extent in meat, kidney, liver, fish, and milk as well as dairy products (Nollet and Toldra, 2012).

Group B vitamins belong to organic substances of different chemical structure, each of them has important individual feature also in the action of human body (Lukaski, 2004 and Carey and Sundberg, 1990).

Taking into account the great amount of vitamins, multivitamins and multidrug formulations based on group B vitamins produced by various manufacturers, the production of vitamins, quality control, validity periods, and use in medical practice are not possible without the careful control over their manufacture, storage and use (Blake, 2007 and Livaniou et al, 2000). Therefore, it seems natural in the last decades, areal boom in the search for novel methods of analysis of group B vitamins in various objects was observed (Okbamichael and Sañudo, 2004 and Markopoulou et al., 2002). The most frequently used methods are chromatography (Capo et al., 2000, Leporati et al., 2005 and Marszałł et al., 2009) chemiluminescene (Wang et al., 2007 and Kumar et al., 2009) spectrophotometry (Esteve et al, 1995 and Liu et al.,

2002) capillary zone electrophorisis (Su et al., 2004 and Chen et al., 2000) and flow-injection analysis with various types of signal registration (Pérez et al., 2005, Portela et al., 2004 and El-Gindy et al, 2003), some of vitamins can be determined simultaneously (Samadi et al, 2008, El-Gindy et al., 2004 and Zhang et al., 1990) rather than electrochemical methods are used.

Various potentiometry and voltametametry methods have been used to determine vitamins in pharmaceutical formulations (Vaze and Srivastava, 2008 and Mostafa, 2003), foods (Kotkar et al., 2007), biological items (El-Maali, 1992), shampoos and creams, and multivitamin formulas (Wang and Tseng, 2001).

Effective separation and quantification of the four water-soluble vitamins was achieved in less than 15 min, Thomas et al. (2008) reported that the running time was about 60 minutes by using a C18 column (250x4. 6mm) with 5 µm of particle size and with gradient elution of mobile phase methanol–15mM 1-hexane sulphonic acid sodium salt solution pH 3,00 for determination of thiamine hydrochloride, riboflavin, nicotinamide and pyridoxine hydrochloride.

The aim of present paper is to develop sensitive, rapid and simple ion-pair RP-HPLC method with UV/VIS detection for determination of B-group vitamins: Thiamine hydrochloride, riboflavin, niacinamide, pyridoxine hydrochloride in pharmaceutical formulation (tablet).

It would be advantageous in the routine analyzed the four water-soluble vitamins in tablet preparations, if they could be determined simultaneously in a single chromatographic run.

Materials and Methods

Instrument

The HPLC system (shimadzu-japan) consist of control unit (scl-10-A vp) , oven (CTO-10A vp) and two types of detectors: Ultra violet Detector (SPD-10AV vp), fluorescent Detector (RF-10AXL), degassing unit (DGU-14A) isocratic pump, with valve 20-µL loop, (LC-solution) software was used for collected data, Chromatographic analysis was performed on RP-C18 (250 mm x4. 6 mm, 5 µm particle size) column from Knauar (Germany).

Reagents and Materials

The present study describes a sensitive and simple Ion-pair RP-HPLC method with UV/VIS detection for simultaneous determination of B-group vitamins (Table 1) below summarize studied compound.

All chemicals and reagents were of analytical grade and the water was distilled and filtered through a membrane filter 0.45µm.

Thiamine hydrochloride, riboflavin, niacinamide, pyridoxine hydrochloride,(BASF - Germany) were used as working standards. Methanol for HPLC, glacial acetic acid, (Merck), heptanesulphonic acid sodium salt,acetonitrilee (Sigma), were used to prepare the mobile phase and sodium hydroxide 2N (Merck) for adjusting mobile phase pH value.

Dosage form

Vitamin B complex® coated tablet: thiamine hydrochloride (vit.B1)5mg, riboflavin (vit.B2)2mg, pyridoxine hydrochloride (vit.B6)2mg, niacinamide (vit. PP) 20mg.

Mobile phase preparation

Several mobile phases were examined in our search, mixture (water: methanol: glacial acetic acid) ratio (72:27:1 v/v) that contain in every 100 ml 140mg, 5 Mm heptanesulphonic acid sodium salt as the ion pairing reagent were investigated and established.

The best results for the simultaneous determination of B1, B2, B3, B6 vitamins were obtained after adjusting pH to 5 ± 0. 2 by using 2N sodium hydroxide solution, the mobile phase was degassed by filtering through a Milli-Q 0. 45µm pore membrane filter.

Chromatographic Conditions

HPLC analysis was carried out using HPLC equipped with fluorescence. (HPLC-FL) (Shimadzo Technologies, Japan). 20 µL of sample solution was injected into Rp-C18(250 mm x4. 6 mm, 5 µm particle size). A mixture (water: methanol: glacial acetic acid) ratio(72:27:1 v/v) as the mobile phase that contain in every 100 ml 140mg, 5 mM heptanesulphonic acid sodium salt as the ion pairing reagent, the flow rate was 1. 0 mL/min, measurements were made at λ=280 nm.

PROCEDURE

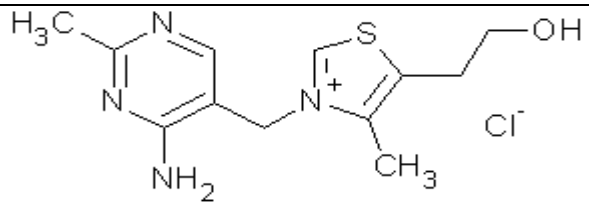
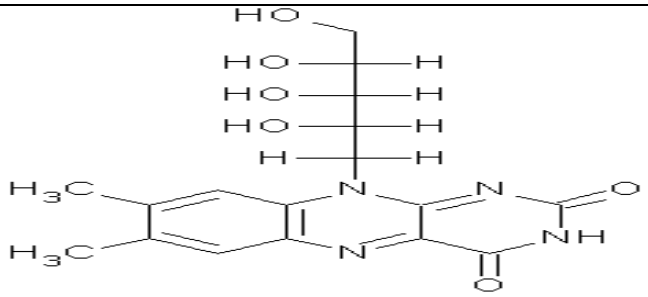
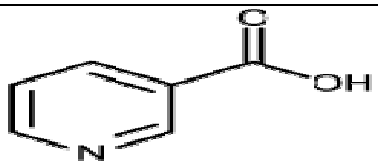
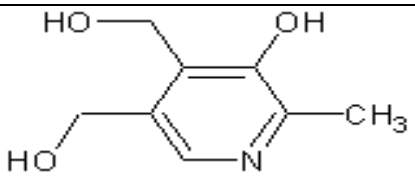
Solvent preparation

Mixture of (water: acetonitrile: glacial acetic acid) with ratio (94 :5 :1 v/v) was selected as suitable solvent for studied materials, due this mixture of solvent dose not absorb light at selected wave length which was 280nm, in addition to all studied vitamins are very soluble in this solvent mixture .

Preparation of Standard Solution

Accurately weighed amounts, 200mg niacinamide, 20mg pyridoxine hydrochloride, 20mg riboflavin, 50mg thiamine hydrochloride, were taken to that

Table 1. chemical structure, IUPAC name and generic name of studied compound.

Chemical structure andIUPAC name	Vitamin and generic name
 (3-[(4-amino-2-methyl-5-pyrimidinyl)methyl-5-(2-hydroxyethyl)-4-methyl thiazolium chloride hydrochloride) **Fig 1: chemical structure and IUPAC name of thiamine hydrochloride**	Vitamin B1 Thiamine hydrochloride
 7,8-dimethyl-10-(2,3,4,5-tetrahydroxypentyl)benzo[g]pteridine-2,4-dione **Fig 2: chemical structure and IUPAC name of riboflavin**	Vitamin B2 riboflavin
 (3-pyridine carboxamide) **Fig 3: chemical structure and IUPAC name of niacinamide**	Vitamin B3 (pp) niacinamide
 (5-hydroxy-6-methyl-3,4-pyridine dimethanol hydrochloride) **Fig 4: chemical structure and IUPAC name of Pyridoxinehydrochloride**	Vitamin B6 Pyridoxinehydrochloride

25. 0 flask, suitable amount of selected solvent was added, the flask was immersed in a hot water bath maintained at 65 – 70C° for 10 minutes and mixed on a vortex-mixer until complete solubility, cooled to room Temperature, the flask was made up to the mark with same solvent, 5 ml of this solution was transferred into a 50 ml volumetric flask, diluted to the mark with the same solvent and filtered through 0. 45 µm membrane filter, clear filtrate was used, the filtrate can be used within 3 hours of filtration.

Preparation of Sample Solution

Sample preparation of B1, B2, B6 and pp. Twenty tablets were weighed and triturated to a fine powder. The average mass of one tablet was

transferred into a 25 ml volumetric flask and solution was added. The flask was immersed in a hot water bath maintained at 65 – 70C° for 10 minutes and mixed on a vortex-mixer until complete solubility, cooled to room temperature, the flask was made up to the mark with same solvent and filtered through 0.45 µ membrane filter. The obtained concentrations for standard solution thiamine hydrochloride (vit.B1) riboflavin (vit.B2), pyridoxine hydrochloride (vit.B6) and niacinamide (vit. PP), compare to the labeled amount per tablet (Table 2).

Table 2. Prepared standard solution concentrations comparing to the labeled dosage form concentration.

Prepared standard solution concentration mg/ml	Labeled concentration per tablet mg/tab	Vitamin
0. 20	5 mg	B1 (Thiamine hydrochloride chloride)
0. 08	2 mg	B2 (riboflavin)
0. 08	2 mg	B6(Pyridoxine hydrochloride)
0. 80	20 mg	PP(niacinamide)

HPLC procedure

Prior to injection into the chromatographic system, all analytical solutions were degassed by sonication. All the prepared sample solutions were first chromatographed to ensure that interfering peaks were not present. 10 µl and 100 µl aliquots of the standard solutions and sample solutions were injected. Triplicate injections were made for each solution.

Results and Discussion

The aim of this study was to develop a simple, accurate and precise HPLC method for simultaneous determination and separation of four vitamin niacinamide (vit. PP), pyridoxine hydrochloride (vit. B6), riboflavin (vit. B2) and thiamine hydrochloride (vit. B1) in pharmaceutical formulation (tablets). The method was developed based on mixture (water: methanol: glacial acetic acid) ratio (72:27:1 v/v) as the mobile phase that contains in every 100 ml 140mg 5mM heptanesulphonic acid sodium salt as the ion pairing and octadecyle column ODS c18(250x4. 6,5µm) with UV detector 280 nm. Vitamins separation has various characteristics; there were acids, bases or neutral compounds under certain circumstances. Samadi et al. (2008) also reported similar findings.

At the RP-HPLC, the neutral compounds would be retained on column depend of their polarity, but the ionic compounds would be eluted spontaneously. A mixture of ionic and neutral compounds could be separated by RP–ion-pair chromatography. The ionic compounds were pairing with counter ion and distributed between mobile and stationary phase as a non-ionic molecule, by using an alkylsulphonate as a counter ion, the cation such as thiamine could be a non-ionic molecule and retained on column because of lipophylicity of alkyl chain. Solution of 1-hexane sulphonic acid sodium salt was used to decrease retention time. The typical chromatogram of standard studied compounds was shown (Fig. 5). El-Gindy (2003) also reported similar findings..

The optimization procedure included studies concerning composition of mobile phase, flow-rate and temperature. After establishing optimal conditions for separation, selectivity, linearity, precision, limit of detection and limit of quantification were determined, chromatographic parameters ie, capacity factor, selectivity factor, resolution factor and factor symmetry, were calculated on the basis of the experimentally obtained, values of retention times and width peaks for all the investigated B-complex vitamins. Under the described experimental conditions, there is no scientific difference between the values of retention times for samples and standards of studied

Table 3. Peaks summary statics for standards and samples of studied compound.

vitamin	RT (min)	AUC
B1 (standard)	765 .8	1611889
B1(sample)	729 .8	1619110
mean	743 .8	207 .1617999
Std. . Dev	019 .0	739 .1570
RSD%	22 .0	10 .0
B2 (standard)	151 .11	6810166
B2 (sample)	101 .11	6828197
mean	126 .11	430 .681918
Std. . Dev	035 .0	695 .12749
RSD%	31 .0	19 .0
PP (standard)	318 .3	2164957
PP (sample)	314 .3	2165263
mean	316 .3	937 .2165109
Std. . Dev	003 .0	869 .216
RSD%	08 .0	01 .0
B6 (standard)	715 .4	3587391
B6 (sample)	705 .4	3593818
mean	710 .4	207 .3590604
Std. . Dev	007 .0	235 .4544
RSD%	15 .0	13 .0

compounds (Table 3) showed peaks summary statics (mean, Std Dev, RSD%) which indicates suitability of our selected chromatographic conditions for separation and determination B group vitamins. Kumar et al. (2009) also advocated the similar findings of vitamins.

The values of selectivity factor were 1.5 for nicotinamide / pyridoxine hydrochloride/,1. 4 thiamine hydrochloride/pyridoxine hydrochloride and 1. 6 for ribofalavin/ thiamine hydrochloride. The resolution factors Rs between the chromatographic peaks were calculated from the equation Rs= 2 (t2 – t1)/(W1 +W2), where t2, t1 are the retention times of the two components and W1, W2 are the peak widths at the base of the two respective peaks: 4.2 for pyridoxine hydrochloride/nicotinamide, 4. 6 for riboflavin/pyridoxine hydrochloride and 8. 4 for thiamine hydrochloride/riboflavin. Representative chromatogram of the working standard solution of B1, B2, PP and B6 was presented (Fig 5). Wang and Tseng (2001) also advocated the similar findings.

The assay was selective, no significant interfering peaks were observed at the retention times of the vitamins. All excipients eluted at different times and did not interfere with the analyzed compounds. The representative chromatogram of the sample solutions of vitamin B1, B2, PP, B6 presented (Fig 6). Zhang et al. (1990) also reported sensitive membrane electrodes for the determination of vitamin B1 and vitamin B6.

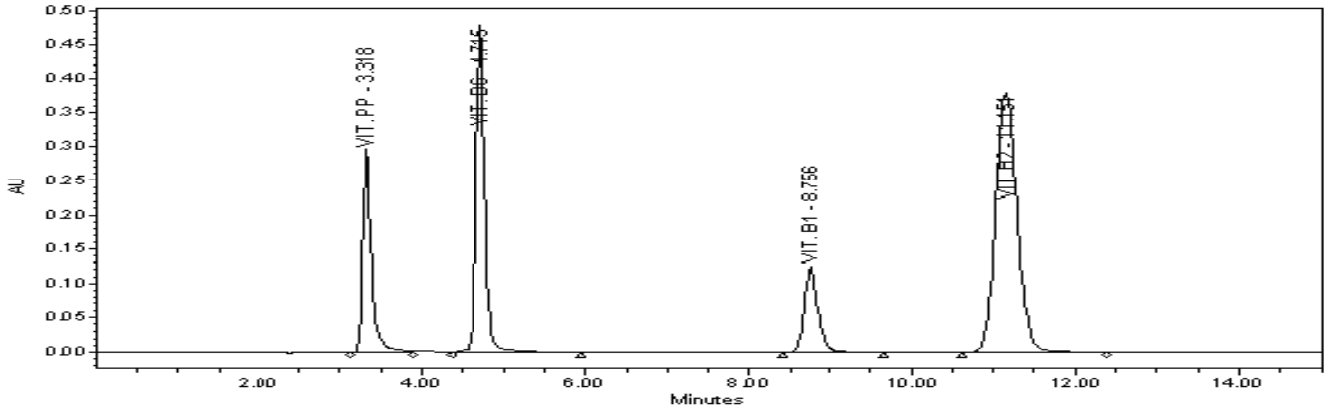


Fig 5: Representative chromatogram of the standard solution of vitamin B1, B2, PP, B6.

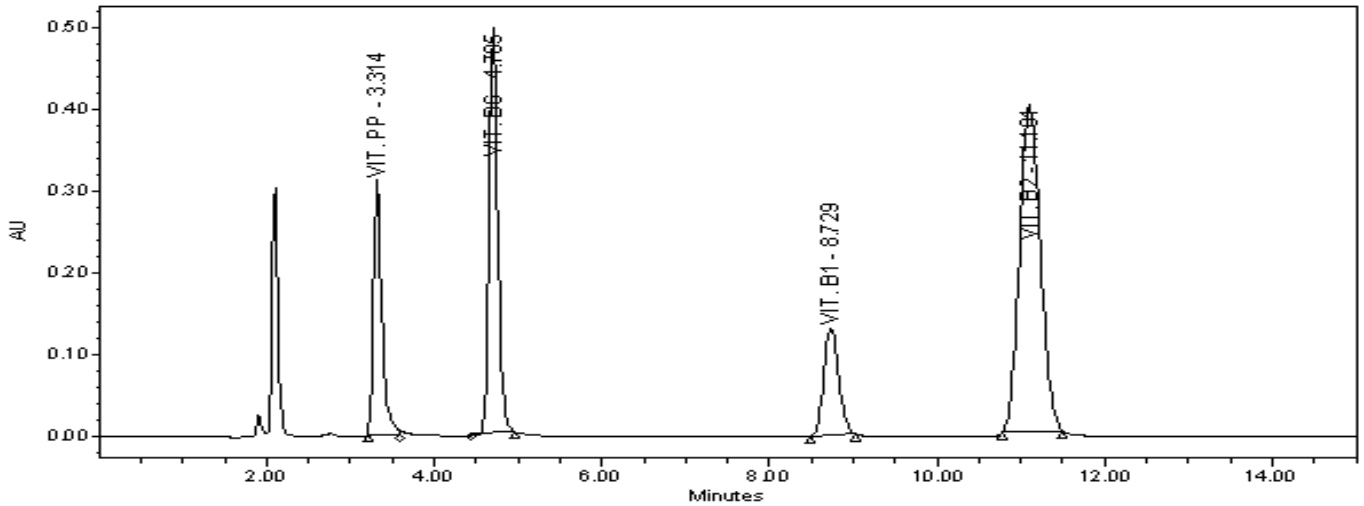


Fig 6: Representative chromatogram of the sample solution of vitamin B1, B2, PP, B6.

The linearity of the method was determined by injecting five solutions of concentration between 50%and 150%of the expected concentration, analysis were performed in triplicate to determinate the linearity of the assay. Good linearity's were obtained with correlation coefficients above 0. 99. The important parameters of calibration curves, ie, slope(a), intercept (b)and correlation coefficient (r) were presented (Table 4).

Table 4. The important parameters for the calibration curves.

vitamin	y = ax +b	r
B1	y = 33472. 3 x + 659. 06	0. 9997
B2	y = 4163. 216 x – 1118	9994 .0
B pp	y = 1570. 58 x + 2484. 68	0. 9995
B6	y = 30344. 56 x – 2616. 38	0. 9980

The precision of the procedure was checked by analysis of ten working standard solutions (B1 10 µg/ml, PP 40 µg/ml, B6 4 µg/ml and B2 4 µg/ml). The RSD values 1. 3 %; 0.6 %; 0.1 %and 0.4 % for B1, PP, B6 and B2, respectively, were indicative of the satisfactory repeatability and thereby the precision of the system. The limit of detection (LOD) and limit of quantification (LOQ) for the investigated vitamins were experimentally determined (Table 5). Chen et al. (2000) advocated similar findings of vitamins.

Table 5. Limits of detection (LOD) and limits of quantification (LOQ).

vitamin	LOD/(µg/ml)	LOQ/(µg/ml)
B1	3125 .0	6250 .0
B2	1250 .0	2499 .0
B pp	3125 .0	6250 .0
B6	0780 .0	1538 .0

The accuracy was carried out at 80%, 100%, and 120% of specification limit, table 6 shows recoveries mean, concentrations found mean and relative standard deviation mean for each vitamin, good accuracy and reproducibility of used method were obtained. El-Maali (1992) also reported determination of vitamin B9.

Table 6. Results of the determination of B-group vitamins in B complex® coated tablets.

Vitamin	Amount in B complex® tablet	found	Recovery %	RSD %
B1	5. 0 mg (3. 5–5. 5 mg)	4. 520 mg	4 .90	2 .1
B2	2. 0 mg (1. 25–2. 75 mg)	2. 104 mg	2 .105	4 .0
B pp	20. 0 mg (17. 0–23. 0 mg)	21. 38 mg	9 .106	7 .1
B6	2. 0 mg (1. 25–2. 75 mg)	2. 104 mg	2 .105	0 .2

Conclusion

It was concluded that simultaneous determination of the four water-soluble vitamins thiamine hydrochloride, riboflavin, nicotinamide, and pyridoxine hydrochloride was performed on column of c18(250x4. 6,5µm), a mixture of (water: methanol: glacial acetic acid) ratio(72:27:1 v/v) as the mobile phase with flow rate of 1mL/men, the effluent was monitored at 280 nm. The simplicity, rabidity, spicifity and accuracy of the procedure should make it highly desirable for quality control of multivitamin products in the pharmaceutical and health food industries.

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